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Oxidative stress and matrix metalloproteinases in primary pterygium

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Abstract
Aim: Pterygia are common conjunctival degenerations with well-documented risk factors but unclear pathogenesis. A better understanding of the pathogenesis of pterygium could lead to improved surgical outcomes and decreased postoperative recurrence. Oxidative stress is considered a key factor involved in the pathology of many chronic diseases, including ophthalmic complications and inflammatory processes.
We aimed to assess the role of oxidative stress and matrix metalloproteinases (MMPs) in the etiopathogenesis of primary pterygium.
Material and Methods: We prospectively examined 26 eyes of 26 patients (mean age 52.76±14.92 (31-77) years) who underwent pterygium excision surgery with conjunctival autograft (CA) transplantation. Pterygium tissue samples were stored after excision. During the operation, conjunctival autograft tissue samples were obtained from the intact conjunctival tissue at the upper temporal quadrant. Part of the conjunctival autograft tissue was kept for the control group. All conjunctival samples were kept at -80°C until biochemical analysis. Total antioxidant status (TAS), malondialdehyde (MDA), matrix metalloproteinase (MMP)-2, and MMP-9 levels were examined in all samples.
Results: In the pterygium group, mean TAS, MDA, and MMP-9 levels were significantly increased when compared to those of the control group ($p<0.05$). In the pterygium group, mean MMP-2 levels were increased when compared to the control group. However, this was not a significant difference ($p>0.05$).
Discussion: These findings indicate that oxidative stress and MMPs may be effective in the etiopathogenesis of pterygium. As a result of this information, it should be considered that local antioxidant treatments may be effective in preventing pterygium progression or recurrence.

Keywords
Pterygium, MMP-2, MMP-9, MDA, TAS

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Introduction

Pterygium is an ocular surface disease caused by chronic ultraviolet-B (UV-B) radiation exposure. UV-B exposure leads to pterygium growth by increasing oxidative stress and a lot of mediators. Oxidative stress causes structural tissue changes by damaging DNA. Inflammation and neovascularization are effective in the formation of pterygium [1].

Recent studies implicate growth factors, apoptotic mechanisms, extracellular matrix modulators, cytokines, immunological mechanisms, genetic factors and viral infections. UV-B exposure and incidental oxidative stress induce pterygium pathogenesis. Pterygium development occurs due to cell migration and angiogenesis. UV-B and inflammatory mediators upregulate the expression of matrix metalloproteinases (MMPs), which engenders extracellular matrix (ECM) remodeling. ECM remodeling may provide limbal degeneration and pterygium formation. Some MMPs, for example, MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9 increased in pterygium body. ECM remodeling may be a common cause of the invasive mechanism of pterygium [2, 3].

We attempt to investigate the pathogenesis of primary pterygium. The motivation of our article was to explore the relationship between the MMP-2, 9 and total antioxidant status (TAS), malondialdehyde (MDA) amounts in the pathogenesis of primary pterygium.

Material and Methods

This study was approved by the Ethics Committee of Ankara Numune Education and Research Hospital (Number: E-18-2443), the tenets of the Declaration of Helsinki were observed. We included our prospective study of 26 eyes of 26 consecutive patients (age range 31-77 years, mean age: 52.76±14.92 years) who need surgical pterygium excision. Seventeen patients were female and nine were male. Healthy conjunctival tissue samples were obtained from those 26 eyes of superotemporal conjunctival autograft tissue. Pterygium tissue samples were also derived from those eyes.

Exclusion Criteria:

Recurrent pterygium, glaucoma, history of previous ocular surgery or trauma.

Surgical Technique:

Pterygium excision was performed under local anesthesia. Peribulbar 2% lidocaine was injected preoperatively for anesthesia. Pterygium was dissected from the cornea using a 45 degrees knife. The pterygium was dissected up to 4 mm from the limbus. Dissection was done in this region until bare sclera was seen below. All pterygium tissue was removed. Haemorrhages were tamponade with compression. Cautery was denied.

The graft was taken from superotemporal region. The conjunctival graft was dissected using Westcott tenotomy scissors. The graft was superimposed on the bare sclera and was placed in the limbus-limbus position. Each graft was sutured with 8/0 Vicryl sutures. Dexamethasone ophthalmic suspension, ofloxacin 0.3% ophthalmic suspension and preservative-free artificial tears were applied four times daily for 1 month after surgery.

Intraoperatively, pterygium tissue (2mm x 2mm) was excised and collected as pterygium samples. Autograft tissue samples (2mm x 2mm) were obtained from the superotemporal bulbar conjunctiva of the same eyes for the control group. All conjunctival samples were kept at -80 °C until biochemical analysis.

Biochemical Analyses:

MDA, TAS, MMP-2, and MMP-9 levels were analyzed in all samples.

The TAS measurements were made using Rel Assay Diagnostics commercial kit. The basis of the assay is to incubate 2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS), with the ferryl myoglobin radical formed by the activation of a peroxidase (metmyoglobin) with H_2O_2 to produce the radical cation ABTS+. It has an approximately balanced blue-green color, measured at 600 nm. Antioxidants in the affixed sample cause suppression of this color reproduction are comparative to its concentration. MDA (the lipid peroxidation end product) levels were determined using the thiobarbituric acid (TBA) test based on the spectrophotometric measurement of the concentration obtained from the end product of the reaction between lipid peroxides and TBA.

MMP-2 and MMP-9 concentrations in the samples were analyzed using commercial 96-well enzyme-linked immunosorbent assay (ELISA) kits.

Statistical Analysis

Statistical analyses were completed using the statistical package SPSS for Windows, version 22.0 (SPSS, Chicago, Illinois, USA). The nonparametric Wilcoxon signed test was used in the statistical analysis. $p<0.05$ was considered statistically significant. Data were introduced as the mean±SD.

Results

The mean TAS, MDA, and MMP-9 levels were significantly higher in the pterygium group when compared to the control group ($p<0.05$). The mean MMP-2 levels were increased in the pterygium group when compared to the control group. However, this was not a significant difference ($p>0.05$) (Table 1).

Table 1. TAS, MDA, MMP-2, and MMP-9 levels in pterygium and the control group (Mean±SD)

Groups/Parameters	Pterygium (n=26)	Control (n=26)	p
TAS mM/mg protein	1.39±0.5*	1.07±0.43	0.006
MDA nmol/mg protein	4.461±1.249*	3.379±1.057	0.006
MMP-2 ng/mL	45.866±17.741	37.923±27.801	0.118
MMP-9 ng/mL	6.456±1.104*	2.922±1.506	0.000

*p<0.05, compared to the control group.

Discussion

The etiopathogenesis of pterygium is controversial. UV is an effective factor in pterygium formation. However, causes such as immune-inflammatory reactions, oxidative stress, virus infections and genetic factors have also been reported to be associated with pterygium. Environmental stimuli trigger the secretion of proinflammatory cytokines like interleukin (IL)-1, IL-6, IL-8, IL-17, IL-23, and tumor necrosis factor (TNF)-α from the ocular surface epithelium and inflammatory cells. These

cytokines promote extracellular matrix remodeling, fibroblast proliferation and angiogenesis in pterygium body. TNF-α and IL-1β increase the mRNA and protein expression of MMP-1 and MMP-3 in cultured pterygium fibroblast cells. Additionally, MMP-2, MMP-3, MMP-8, MMP-9, MMP-10, and MMP-13 have also recently been shown to be overexpressed in pterygium tissue [4-8].

Our present results showed that MMP-2 and MMP-9 levels increased in pterygium tissue but only MMP-9 levels were statistically significant. Contrary to our study, there are also studies where MMP-9 could not be detected in primary and recurrent pterygium tissues. However, they reported an increase in MMP-1 and MMP-3 [9]. Yang et al. reported that the expression of MMP-2 and MMP-9 by pterygium fibroblasts increased after the progression of pterygium [10].

We also measured the TAS and MDA levels in pterygial and normal conjunctival tissues. We found that the mean TAS and MDA levels were significantly higher in pterygium tissues. These increased levels were attributed to local oxidative stress. Some authors investigated that in the pterygium group, MDA level and catalase (CAT) activity were higher, whereas superoxide dismutase (SOD) activity was lower when compared to control groups. In the face of the significant increment in CAT activity, the MDA level was still superior in the pterygium group, demonstrating an outward growing oxidative damage [4].

Kormanovski and coworkers investigated decreased TAS in recurrent pterygium tissue. They attributed to the decreased antioxidant defense playing role in the recurrence of pterygium. They also showed that TAS levels were significantly increased in the primary pterygium group [11]. We think that the increased TAS in pterygium tissue indicates the antioxidant defense mechanisms of the primary pterygium from increased oxidative stress.

Kau et al. demonstrated abundant immunoreactivity of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in pterygium tissues and elevated levels of 8-OHdG in the DNA of pterygium tissues compared to normal conjunctiva. We understand from these findings that when DNA is oxidatively damaged, it may cause pterygium development [12]. These findings show new horizons of the pathogenesis of pterygium. We must consider this in the avoidance and treatment of pterygium.

Maxia and colleagues have proven oxidative stress may be associated with activation of survivin expression, this may play an important role in the development of pterygium by inducing hyperproliferation in tissue [13].

Pterygium is a benign ocular surface disease that often tends to progress and recur. Myofibroblasts in pterygium tissue are resistant to cell death and are responsible for extracellular matrix synthesis through tissue remodeling. Although many surgical methods have been tried to prevent pterygium recurrence, it may be more effective to eliminate the factors in the etiology [14].

Increased levels of antioxidant potential and MDA in the primary pterygium confirm that oxidative stress plays an important role in the pathogenesis of pterygium. In this study, we demonstrated that MMP-2 and MMP-9 may be causative factors in formation and invasion of pterygium. Oxidative stress and MMPs may be the leading causes in the formation and progression of pterygium.

Conclusion

In our study, the pterygium tissue of the patients was compared with the conjunctival tissue in the same eye that was not exposed to ultraviolet light and external factors. In this respect, we think that the results we obtained provide important data for pterygium. These results should provide ideas to find new treatments to prevent pterygium formation and progression.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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